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# **The Importance of Mosquito Behavioural Adaptations to Malaria Control in Africa**

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### **Abstract**

Over the past decade the use of long-lasting insecticidal nets (LLINs), in combination with improved drug therapies, indoor residual spraying (IRS) and better health infrastructure, has helped reduce malaria in many African countries for the first time in a generation. However, insecticide resistance in the vector is an evolving threat to these gains. We review emerging and historical data on behavioural resistance in response to LLINs and IRS. Overall the current literature suggests behavioural and species changes may be emerging, but the data are sparse and, at times unconvincing. However, preliminary modelling has demonstrated that behavioural resistance could have significant impacts on the effectiveness of malaria control. We propose seven recommendations to improve understanding of resistance in malaria vectors. Determining the public health impact of physiological and behavioural insecticide resistance is an urgent priority if we are to maintain the significant gains made in reducing malaria morbidity and mortality.

1 Long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are currently the key  
2 components of vector management strategies used for the control of malaria (Roll Back Malaria  
3 Partnership 2005). Over the past decade the use of LLINs, in combination with improved drug  
4 therapies, IRS and better health infrastructure, has helped reduce malaria in many African countries  
5 for the first time in a generation (O'Meara et al. 2010, World Health Organization 2010). Malaria  
6 mortality has declined since 2000 by 25% globally and 33% in sub-Saharan Africa (World Health  
7 Organization 2011a). These remarkable successes have created unprecedented optimism about  
8 reaching the malaria reduction targets outlined in the Global Malaria Action Plan (Roll Back Malaria  
9 Partnership 2008) and, ultimately, for the local elimination of malaria; however, there is a growing  
10 threat to these gains in the form of insecticide resistance.

11  
12 Evolution of resistance to the chemotherapeutic is a common outcome of effective (and ineffective)  
13 vector or parasite control programmes. While this is often viewed as a failure of the program, it is  
14 better regarded as an almost inevitable consequence since history has repeatedly shown that  
15 intensive interventions lead to the emergence of physiological (biochemical) resistance due to the  
16 high selective pressure exerted on the targeted population. The emergence of resistance in the  
17 vector has not only developed against all four classes of insecticide licensed to control adult  
18 mosquitoes for public health purposes (Ranson et al. 2011, World Health Organization 1970), but  
19 also in the malaria pathogen against the most widely used antimalarials, starting with chloroquine,  
20 the standard drug of treatment during the Global Malaria Eradication Campaign (GMEC) (Najera  
21 1999). Indeed, resistance was one of the reasons cited for ending the GMEC in the late 1960s (Najera  
22 1999). At that time resistance to DDT had developed in 14 anopheline species. The recent emergence  
23 of artemisinin drug resistance in South East Asia (Phyo et al. 2012, World Health Organization 2011b),  
24 makes clear that this is not simply a problem of the past, nor one confined to insecticides.

1 The finding of widespread physiological resistance to pyrethroids in *Anopheles gambiae*, the major  
2 vector of malaria in Africa (Ranson et al. 2011), is a major public health concern because pyrethroids  
3 are the only insecticides currently used for treating bed nets. Results from experimental hut studies  
4 in West Africa demonstrate a marked reduction in mosquito mortality in areas with high levels of  
5 physiological resistance (N'Guessan et al. 2007). Of course, in the absence of effective chemical  
6 control, intact bed nets still provide barrier protection against biting mosquitoes (Clarke et al. 2001,  
7 Snow et al. 1988); however, nets become worn and it is likely that torn or holed treated nets provide  
8 inadequate protection in areas where pyrethroid-resistant vectors are common (Irish et al. 2008,  
9 N'Guessan et al. 2007). This has recently been confirmed in studies at the same locations as the  
10 experimental hut trial (Asidi A. et al. 2012). In households in the areas where resistant mosquitoes  
11 were common there were high rates of blood-feeding and freshly treated nets provided no  
12 protection once holed. In contrast, sleeping under a holed bed net in the location where susceptible  
13 mosquitoes were common decreased the odds of being bitten by 66% and the majority of  
14 mosquitoes were killed by the treatment.

15  
16 At present there are 40 malaria-endemic countries reporting resistance to insecticides, most to  
17 pyrethroids (WHO Global Malaria Programme 2012). Multiple insecticide resistance is also common,  
18 with some regions having resistance to all four insecticide classes used in public health (Ranson et al.  
19 2009). Although evidence is currently lacking that this level of resistance is impeding malaria control,  
20 most experts expect that current vector control efforts will soon be compromised unless strategies  
21 are implemented to manage the resistant vectors. It is estimated that more than half of the benefits  
22 gained from the current coverage of LLINs and IRS in Africa would be lost if pyrethroids lose their  
23 efficacy, resulting in approximately 120,000 additional deaths per year (WHO Global Malaria  
24 Programme 2012).

1 As a consequence of this growing threat, the World Health Organisation Global Malaria Programme  
2 have published a Global Plan for Insecticide Resistance Management (GPIRM) (WHO Global Malaria  
3 Programme 2012). This strategy focuses solely on physiological resistance in malaria vectors.  
4 However, it is likely that behavioural resistance may also develop in response to insecticide exposure.  
5 Furthermore, how the behaviour of physiologically resistant vectors might differ in comparison to  
6 their sensitive counterparts is very poorly known.

7  
8 Behavioural resistance refers to any modification to mosquito behaviour that facilitates avoidance or  
9 circumvention of insecticides. The contribution of behavioural changes in agricultural pests to  
10 insecticide/pesticide resistance has been long acknowledged (Gould 2010, Sparks et al. 1989), with  
11 theoretical studies providing valuable information to inform and improve management practice s  
12 (Castillo-Chavez et al. 1988, Gould 1984). In comparison, determining if behavioural adaption in  
13 vectors may be of medical importance has lagged behind. Perhaps the best-documented behavioural  
14 change in malaria vectors, and the biggest concern, is the development of an early, outdoor feeding  
15 phenotype among anopheline populations in areas of extensive indoor insecticide use. These  
16 mosquitoes may circumvent LLIN and IRS control through preferential feeding and resting outside  
17 human homes and being active earlier in the evening before people have gone to sleep. In addition,  
18 there are a variety of other changes in vector behaviour such as increased zoophagy that may evolve  
19 in response to intensive interventions. Part of the reason for the lack of information about  
20 behavioural resistance is that it is harder to investigate using relatively simple exposure assays, and  
21 far more difficult to monitor in field populations, compared to physiological resistance (Ferguson et  
22 al. 2010, Takken 2002).

23  
24 Both physiological and behavioural resistance to insecticides may be determined by a limited number  
25 of major genes or be affected by a relatively large number of genes of small effect. The genetic basis  
26 of resistance affects the dynamics of spread as well as the ease with which molecular markers of

1 resistance can be developed. Phenotypes caused by single gene mutations generally demonstrate an  
2 exponential increase in frequency, where much of the initial stage of spread occurs at very low, near-  
3 undetectable gene frequencies, prior to a period of rapid amplification to high frequencies. In  
4 contrast, phenotypes based on standing genetic variation in many genes typically have a different  
5 dynamic: their spread is generally described by an immediate and sustained change in their  
6 phenotypic distributions.

7  
8 One cannot predict *a priori* which model will apply to a specific trait. Physiological resistance may  
9 occur through single mutations, for example *knock-down resistance (kdr)* in the sodium channel  
10 protein targeted by pyrethroids, but may also arise through altered levels of detoxifying enzymes  
11 such as P450s and esterases, whose expression levels may well be modulated by variation in many  
12 genes, making it a quantitative genetic trait (Ranson et al. 2004, Wondji et al. 2009). Similarly,  
13 behavioural changes are often regarded as complex, quantitative genetic traits but there are  
14 instances of a single gene mutation in insects having large effects on behaviour. For example,  
15 polymorphisms in the phosphoglucose isomerase (*pgi*) gene are associated with differences in  
16 butterfly dispersal rates as well as other phenotypic traits (Niitepold et al. 2009), while major  
17 mutations in some *Drosophila* circadian rhythm genes can affect their daily behaviour cycles  
18 (reviewed in (Sokolowski 2001)). Another possible example is single gene mutations encoding  
19 physiological insecticide resistance which also appear to change behaviour through pleiotropic action  
20 that alters repellency (see later discussion). An important research gap is therefore a detailed  
21 understanding of the likely genetic basis of specific behavioural resistance traits, and how  
22 surveillance programmes should be implemented to best monitor changes in these traits.

23  
24 Here we review emerging and historical data on behavioural resistance in response to LLINs and IRS  
25 in an effort to understand better the biology underlying the field observations and highlight areas in  
26 need of further research. The data reviewed specifically focuses on the *Anopheles* vectors of malaria,

with an emphasis on sub-Saharan African species where much of the behavioural research has been conducted. The predominant species are *Anopheles gambiae sensu lato* and *An. funestus*. *Anopheles gambiae* s.l. is a species complex consisting of several closely related sibling species including *An. gambiae sensu stricto*, *An. arabiensis*, *An. melas*, *An. merus*, *An. quadriannulatus* Species A, *An. quadriannulatus* Species B and *An. bwambae*. There are a number of secondary vectors contributing to malaria transmission in sub-Saharan Africa which we do not consider (Antonio-Nkondjio et al. 2006).

## **Evidence for impact of indoor insecticides on mosquitoes**

### ***Vector abundance***

The insecticides used for LLINs and IRS exert their effect on the vector population in three ways: toxic chemical action, spatial repellency/deterrence and contact irritancy (Box 1) (Lines J. D. et al. 1987, Smith A. and Webley 1968, Takken 2002). The relative importance of each of these in determining how an insecticide works is dependent not only on the chemical and concentration used, but also on the mosquito species (Chareonviriyaphap 2012, DeZulueta et al. 1963, Grieco et al. 2007) and the application methods (e.g. IRS versus LLINs). The non-toxic chemical effects are highly relevant when assessing the impact of physiological resistance since it is the interaction between toxicity and behaviour that determines the level of insecticide uptake and ultimately the probability that the insect dies.

Data collected from experimental hut studies indicate that bednets treated with pyrethroids and walls sprayed with DDT dramatically increase the rate at which African mosquitoes leave huts and reduce the number of blood-fed mosquitoes compared to untreated controls (Asidi A. N. et al. 2005, Chandre et al. 2010, Lines J. D. et al. 1987). This outcome suggests that these chemicals are contact irritants.



1  
2 The evidence for spatial repellency, where mosquitoes are deterred from entering the house, is  
3 equivocal for treated nets, with some studies finding no reduction in the rate of entry (Chandre et al.  
4 2010, Kirby et al. 2008, Malima et al. 2008, N'Guessan et al. 2010) while others reported significant  
5 decreases (Asidi A. N. et al. 2005, Lindsay et al. 1991a, Lines J. D. et al. 1987). In less controlled  
6 settings, short-term use of treated nets did not appear to impact the number of *An. gambiae* s.l.  
7 entering houses (Mathenge et al. 2001), but instead acted as a contact irritant that increased exit  
8 rates, particularly of unfed mosquitoes, resulting in fewer mosquitoes resting indoors (Akogbeto et  
9 al. 2011, Mathenge et al. 2001, Quinones et al. 1998). Studies with IRS employing DDT suggested that  
10 the compound had some spatial repellence that may reduce mosquito entry into the house  
11 (Dezulueta et al. 1963, Roberts et al. 2000, Smith A. and Webley 1968). The level of deterrence  
12 reported for some experimental hut trials based on the number of mosquitoes collected indoors is  
13 sometimes confounded by the fact that mosquitoes entering the hut can leave by the same opening.  
14 This means that unless mosquito movements are carefully recorded through all openings, reductions  
15 in the rate of entry cannot be distinguished from unrecorded or higher rates of departure (Silver  
16 2008).

17  
18 Whatever the precise mechanism, the large-scale use of LLINs or IRS frequently results in a major  
19 reduction in the abundance of vectors, often referred to as the 'mass community effect' (Hawley et  
20 al. 2003). This effect is the basis for the universal coverage advocated by Roll Back Malaria (RBM)  
21 where the goal is that 80% of people at risk from malaria are protected by vector control methods,  
22 primarily LLINs and IRS (Roll Back Malaria Partnership 2005). Community surveys comparing villages  
23 with and without LLINs, or changes pre- and post-intervention show decreased abundance of indoor  
24 resting mosquitoes (Bayoh et al. 2010, Lindblade et al. 2006, Mbogo et al. 1996), feeding mosquitoes  
25 (Trape et al. 2011) and larvae (Bayoh et al. 2010).

## 1 **Mosquito behaviour**

2 There are a number of possible impacts that insecticide use indoors could have on mosquito  
3 behaviour including changes in biting phenology and the frequency of endophagy. All anopheline  
4 vector species predominantly feed at night. One of the consequences of large-scale indoor  
5 insecticide use is the potential selection for vectors that feed on people earlier in the night while  
6 they are outdoors. Exophily was one of the reasons cited for why IRS (when used in isolation) failed  
7 to reduce malaria parasite rates substantially in the Garki project in northern Nigeria (Molineaux and  
8 Gramiccia 1980). Here large-scale use of IRS with propoxur, a carbamate insecticide, in an area of  
9 high transmission (Entomological Inoculation Rate (EIR) = 18-145 sporozoite-positive bites per person  
10 each year) resulted in a 90% reduction in vectorial capacity, but only reduced the parasite prevalence  
11 for *Plasmodium falciparum* by 25% (the major vector was *An. gambiae* s.l.).

12  
13 *Anopheles arabiensis* populations show a wide range of peak biting times at different sites (Braack et  
14 al. 1994, Dukeen and Omer 1986, Fontenille et al. 1997b, Lemasson et al. 1997, Mattingly 1949,  
15 Yohannes and Boelee 2012, Yohannes et al. 2005), with some of this variation being explained by  
16 season (Tirados et al. 2006). One possible explanation for the remaining variation is that peak biting  
17 times may reflect the historical use of insecticides. Most interventions against this vector have  
18 involved IRS which kills endophilic but not exophilic mosquitoes. Exophilic mosquitoes that are  
19 strongly anthropophilic need to feed early in the evening when humans are readily available  
20 outdoors. DDT has been used for IRS in Ethiopia for the last 40 years and there is some evidence in  
21 this country for increase exophily (Figure 1) (Biscoe et al. 2005, Tirados et al. 2006, Yohannes and  
22 Boelee 2012, Yohannes et al. 2005). A clearer example of selection for early feeding comes from  
23 extensive indoor spraying of DDT to control *Anopheles farauti* in the Solomon Islands (Taylor 1975).  
24 Prior to IRS the peak biting time for *An. farauti* was early evening, declining gradually until early  
25 morning. After extensive IRS the biting activity in the late evening and early morning almost  
26 disappeared, with most occurring in the early evening (Figure 2). While these examples demonstrate

1 clear shifts in biting behaviour, this response to indoor spraying has not been found everywhere. For  
2 example, in northern Sudan where indoor DDT spraying had been used for the 8 years, an  
3 entomological survey found 'standard' biting cycle persisted in *An. arabiensis* (Figure 1) (Dukeen and  
4 Omer 1986).

5  
6 There are also mixed reports of the evolution of behaviour in response to bed net deployment.  
7 Increased exophagy by *An. gambiae* s.l. in response to LLIN use has been reported in Kenya (Mbogo  
8 et al. 1996) but not Tanzania (Russell et al. 2011), while *An. funestus* exophagy increased significantly  
9 in Tanzania (Russell et al. 2011). Inside houses, the proportion of *An. gambiae* s.l. and *An. funestus*  
10 feeding before 2200 h increased, though peak biting still occurred after midnight (Mbogo et al. 1996,  
11 Russell et al. 2011, Trape et al. 2011). In addition, the proportion of early feeding mosquitoes  
12 increased following the introduction of LLINs, yet the absolute number of mosquitoes feeding during  
13 this time was generally less. This suggests that the observations may have resulted from failure to  
14 control a smaller population of residual mosquitoes that continued to bite earlier in the night. It  
15 should also be noted that the changes reported for *An. gambiae* s.l. do not account for any potential  
16 shifts in species composition within the complex. That is, the apparent behavioural changes could  
17 simply reflect effective control of a later-feeding member of the complex so that a previously less  
18 abundant species that feeds earlier in the night becomes relatively more dominant. In spite of these  
19 inconsistencies, the limited data available highlights the importance of monitoring for these  
20 behavioural changes in a range of settings, using a robust experimental and/or observational  
21 approach.

22  
23 The difficulty and expense of accurately measuring mosquito behaviour in the field has also limited  
24 the documentation and understanding of behavioural resistance. In one study, Mbogo et al (1996)  
25 reported a reduction in the overall human biting rate from 95 to 34 bites per night, and an increase  
26 in the proportion of mosquitoes feeding outdoors from 1.2% to 30.3% following the introduction of

LLINs. In the control village the biting rate remained constant at about 5 bites per person per night over the intervention period, and the proportion feeding outdoors increased from 2.7 to 20.3%. These results are difficult to interpret and extrapolate as sampling pre-intervention was conducted in five intervention and four control zones, while post-intervention sampling was only conducted in one intervention and one control village. Data collected by pyrethrum spray collections (PSC) from all the villages in the study indicated significant variations between sites meaning the pre- and post-intervention data may not be directly comparable.

Results presented by Russell et al (2011) are equally difficult to interpret. Their study used data from three field collections in Tanzania conducted i) prior to the introduction of LLINs (1997), ii) after 75% of the community used untreated nets (2004) and iii) after 47% of the population used LLINs (2009) (Russell et al. 2011). No estimate of the overall biting rate is presented. However, graphical data suggest there is little difference in the overall biting rate between 2004 and 2009 for *An. gambiae* s.l., while biting rates for *An. funestus* are higher in 2009 compared to 2004. Compelling data are presented for decreasing endophagy rates for *An. funestus* over the time series, but not *An. gambiae* s.l., and a decreasing proportion of mosquitoes attempting to feed between 2100 h and 0500 h in both species (Russell et al. 2011). Interestingly, the trend for decreasing night feeding was consistent across the sample period, even though the coverage of LLINs only significantly increased in the second part of the time series, between 2004 and 2009.

### **Changes in Vector Dominance**

In parts of Africa the massive scale-up of LLIN deployment is associated with an apparent shift in vector dominance from the highly endophilic *An. gambiae* s.s. to the more exophilic *An. arabiensis*. This is seen in western and southern Kenya (Bayoh et al. 2010, Lindblade et al. 2006, Mutuku et al. 2011, Zhou et al. 2011) and Tanzania (Russell et al. 2011), but not Senegal (Trape et al. 2011). Recent data from western Kenya shows an unexplained resurgence in *An. gambiae* s.s. during 2010 (Zhou et

al. 2011). Species composition changes are also reported in coastal Kenya with significantly less *An. gambiae* s.s and more *An. merus* in intervention compared to non-intervention areas (Mbogo et al. 1996). However, as no pre-intervention data are presented, it is not possible to infer whether this difference is a result of an insecticide-induced change in species composition, or a difference in the initial species composition at the study sites. The change in species composition of adult mosquitoes at some sites is mirrored in the corresponding larval populations (Mutuku et al. 2011).

Analysis of data collected at Lupiro village, Tanzania beginning in 2002 shows a significant reduction in the relative proportion of *An. gambiae* s.s. compared to *An. arabiensis* over time leading the authors to conclude that high LLIN usage has dramatically altered the mosquito populations (Russell et al. 2011). However, closer examination of the data sources reveals a number of potentially confounding factors. For instance, the studies use a variety of collection methods including CDC light traps, human landing catches, resting collections and “Ifakara traps”, and some report data from indoor collections only while others represent both indoor and outdoor collections. Also the studies are conducted at different times of the year relative to the wet and dry season, a factor known to differentially affect the abundance, feeding and resting behaviour of the vectors (Cano et al. 2004, Koenraadt et al. 2004, Mutuku et al. 2011, Reddy et al. 2011, Trape et al. 2011, Wanji et al. 2003). Unfortunately several studies did not report when the collection was conducted, and the dry season collections tended to be clustered later in the study period confounding the interpretation of LLIN impact. Large variations (range: 4% - 96%) in the proportion of *An. gambiae* s.s. between sample clusters were also reported in at least one of the studies included in the analysis (Killeen et al. 2007), demonstrating the extreme variability in the ratio of *An. gambiae* s.s. to *An. arabiensis* in the village.

The above example highlights the complexity of assessing the exact impact of LLINs due to the background variability in the vector populations. Indeed a substantial decline in vector numbers in the Tanga region of Tanzania where vector control has not been used on a large scale was reported

1 between 1998-2009 (Meyrowitsch et al. 2011), highlighting the variability of malaria vector  
2 abundance. Relative species composition also varies greatly over time in natural populations as  
3 demonstrated by the unexplained change in dominant species from *An. funestus* to *An. arabiensis*  
4 between 1992 and 1995 in Dielmo village Senegal (Fontenille et al. 1997a), and the strong seasonal  
5 relationship between the relative abundance of *An. gambiae* s.s. and *An. arabiensis* (Dia et al. 2005,  
6 Highton et al. 1979, Lindsay et al. 1991b, Oyewole et al. 2007, Trape et al. 2011).

7  
8 In one series of experimental hut trials *An gambiae* s.s. was controlled more readily by LLINs than *An.*  
9 *arabiensis* despite having similar sensitivity to pyrethroid (Kitau et al. 2012). It is postulated that this  
10 differential mortality may be attributable to the more zoophilic *An. arabiensis* being less persistent in  
11 its attempts to bite the human host through the net than the more anthropophilic *An. gambiae*.  
12 Whatever the reason, the differential mortality provides one explanation for the possible shift in  
13 species ratio from *An. gambiae* s.s to *An. arabiensis* in areas with high coverage of LLINs.

14  
15 Whether the propensity for outdoor biting by individuals of a given species is increasing or there is  
16 merely a residual population of outdoor-biting vectors is debatable, but the consequences of this  
17 change are important. Outdoor biting is difficult to counter with available control methods. Larval  
18 source management, spatial repellents, transgenic mosquitoes and attractive toxic sugar bait could  
19 be used or developed for malaria control, but are either difficult to scale-up in all locations or are  
20 tools that will need much more research before they can be successfully deployed. The development  
21 of these, as well as new tools targeting outdoor-feeding mosquitoes is an urgent priority.

## 22 23 **What are the population dynamic consequences of the continued use of LLINs and IRS?**

24 Changes in vector abundance and species dominance are linked to processes affecting mosquito  
25 population dynamics. The observed abundance of mosquitoes is determined by the interaction of  
26 density-independent and density-dependent processes affecting mosquito survival and fecundity.

1 The nature and action of the density-dependent processes is particularly critical as it sets the mean  
2 abundance about which populations fluctuate. We still know relatively little about *An. gambiae* s.l.  
3 population dynamics but most vector biologists believe that the most important density-dependent  
4 process involves competition amongst mosquito larvae for food (Smith D. L. and McKenzie 2004). A  
5 few studies that have manipulated larval densities in semi-natural breeding sites show mortality  
6 increases relatively linearly with density (Gimnig et al. 2002, White M. T. et al. 2011). Understanding  
7 the location of density-dependence in the mosquito life cycle relative to where insecticides act, as  
8 well as the shape of the mortality-density function, is important as it determines the degree to which  
9 insecticide deaths are compensated for by reduced density-dependent mortality; that is, it  
10 determines the impact of insecticide on vector population density (Hancock and Godfray 2007).

11  
12 Reductions in mosquito abundance can have two further effects on disease transmission mediated  
13 through density dependence. There is evidence that lower larval densities increase survival, increase  
14 adult size and lower development rate. As Lyimo and Koella (Lyimo and Koella 1992) among others  
15 has pointed out, increased size may be particularly pertinent to disease transmission if larger  
16 individuals live longer and so are more likely to survive through the disease latent period.  
17 Longitudinal surveillance data of mosquito size during an LLIN or IRS intervention would address this  
18 question, as would more data about the relationship between larval density and adult size, and adult  
19 size and longevity, in the field. Second, we know that the larval habitats for different members of the  
20 *An. gambiae* complex differ but overlap (Schneider et al. 2000, Service 1973). We do not know if  
21 these differences reflect adaptations to different niches or if different taxa compete with one  
22 excluding the other. If the latter is the case, then reducing the number of one type of mosquito may  
23 lead to competitive release of another. If the two mosquito taxa have different degrees of  
24 exophily/endophily then the ratio of mosquitoes feeding indoors or outdoors may change through  
25 interspecific population-dynamic processes.

1 Finally, the evolution of resistance typically entails fitness costs to the mosquito which are most likely  
2 to be manifest when the insect is stressed, in particular when it is subject to density-dependent  
3 mortality (Kraaijeveld and Godfray 1997). We do not know the extent to which this happens, or  
4 indeed if it happens at all, but it is quite likely that the demographic and genetic dynamics of vectors  
5 are closely intertwined.

#### 7 **How concerned should we be about the future effectiveness of LLINs and IRS?**

8 The key to the prolonged future success of LLINs and IRS is to understand the biological mechanisms  
9 underlying the changes being observed in the field. One possibility is that insecticide interventions  
10 are selecting for a heritable trait, that is, vectors which are genetically programmed to feed early  
11 outdoors. In this situation the effectiveness of LLINs in reducing malaria infection rates will decrease  
12 over time as the susceptible, indoor-feeding vectors are removed from the population, leaving  
13 predominantly the early outdoor feeders. There is clear evidence for a genetic basis for behavioural  
14 differences as the two closely-related species *An. gambiae* and *An. arabiensis* often broadly differ in  
15 their feeding preferences and propensity to rest indoors or outdoors. In contrast, there are limited  
16 data on the role of genetics in behavioural polymorphisms within a species. It has been reported  
17 that there is an association between the 2R inversion polymorphism on chromosome 2 and  
18 differential endophily and endophagy in *An. arabiensis* (Coluzzi et al. 1977), but also that the  
19 preference of individual mosquitoes for a given resting location (indoor versus outdoor) is not  
20 consistent (Lines J.D. et al. 1986), and that *An. arabiensis* shows site fidelity (returning to location of  
21 feeding) rather than host fidelity (McCall et al. 2001). It has also been suggested that feeding  
22 preferences for *An. gambiae* are related to the abundance of potential host species; in environments  
23 where there are many people the proportion of human bloodfed mosquitoes was high, but  
24 decreased when cattle were abundant (White G. B. 1974). However the sporozoite rates were similar  
25 for mosquitoes which were human bloodfed and cattle bloodfed, suggesting that the feeding



1 preferences of *An. gambiae* s.s. are plastic (White G. B. et al. 1972). This overall plasticity in  
2 behaviour allows continued mosquito survival when host species vary in abundance.

3  
4 An alternative hypothesis, and one which presents a more promising outlook for LLINs, is that early  
5 outdoor feeding is a consequence of unsuccessful feeding on the prior evening. In this scenario  
6 mosquitoes retain their inherent feeding preferences (e.g. location, host and time) and in ideal  
7 situations will feed according to these preferences. With widespread LLIN coverage mosquitoes may  
8 not successfully feed indoors, being thwarted by the net barrier or repelled by the insecticide from  
9 the dwelling. Some of these mosquitoes may succeed in their search for a bloodmeal elsewhere,  
10 while others rest outdoors until the following evening at which time they recommence their search.  
11 These vectors may initiate their search soon after dusk and feed opportunistically on any outdoor  
12 hosts they encounter en route to a more preferred indoor feeding location. Under this hypothesis  
13 LLINs will continue to be effective as populations of indoor feeding mosquitoes will be retained,  
14 albeit with declining abundance caused by direct killing or increased mortality associated with  
15 delayed and non-optimal feeding conditions.

16  
17 Direct evidence supporting such a thwarted feeding theory is limited, although a similar model has  
18 been proposed to explain changes in mosquito host-seeking activities after IRS of houses with DDT  
19 (Roberts et al. 2000). Under this hypothesis the proportion of mosquitoes feeding early in the  
20 evening should be correlated with the probability of obtaining a bloodmeal; the lower the probability  
21 of finding the preferred late-evening bloodmeal, the more mosquitoes which feed early in the  
22 evening. Searching for new hosts (either human or animal), feeding on non-preferred hosts and  
23 finding less suitable resting sites are all likely to be associated with increased mortality due to  
24 foraging risk, thus increasing the indirect impact of LLINs on mosquito survival and disease  
25 transmission. Previous modelling results have indicated that endemic disease transmission is highly  
26 sensitive to changes in mosquito survival during searching or feeding (Saul 2003). It is becoming

1 increasingly clear that interventions such as LLINs and IRS are associated with dramatic reductions in  
2 malaria which often exceed that expected based on measured changes in mosquito abundance alone  
3 (Trape et al. 2011). Thus unobserved secondary impacts, other than direct mosquito killing through  
4 toxicity, are likely to be occurring.

5  
6 A critical appraisal and understanding of the biology underlying field observations is urgently needed  
7 to address the questions surrounding the longer-term prospects of current interventions and assess  
8 potential new interventions. We need to be cautious about inferring selection of new behaviour  
9 patterns when mosquitoes show an inherent plasticity in feeding when frustrated in accessing their  
10 hosts. Overall the current literature suggests behavioural and species changes due to LLINs may be  
11 emerging, but the data are sparse and, at times unconvincing and liable to publication bias,  
12 highlighting the need for greater research effort in this area. Only when these issues are better  
13 resolved can the future impacts of LLINs be fully predicted.

14  
15 Modelling studies provide an important way of investigating the impact that physiological and  
16 behavioural resistance could have on disease prevalence; however, such studies are currently limited  
17 by a lack of understanding of the biological processes affecting insecticide resistance, particularly  
18 behavioural resistance. Theoretical predictions of the impact of IRS demonstrated over 30 years ago  
19 that model output is highly sensitive to assumptions regarding the uniformity of mosquito exposure  
20 to the insecticide (Molineaux et al. 1979), but there has been little advance in understanding the  
21 baseline distribution of exposure and if (or how) this changes following insecticide exposure .

22 Resolving these issues will lead to improved models and better information for policy and control  
23 programs. Public health officials would then be able to address the key questions of whether  
24 resistance will compromise the long-term effectiveness of LLINs and IRS and how best to combat the  
25 problem.

1 To demonstrate the potential of mathematical models for investigating behavioural resistance we  
2 have selected one behavioural parameter, exophagy, and investigated its influence on the  
3 effectiveness of LLINS and IRS using two different comprehensive malaria transmission models  
4 (Chitnis et al. 2012, Griffin et al. 2010, OpenMalaria 2012, Smith T. et al. 2008) (see Box 2).  
5 Importantly, both models reach the same conclusion; that the impact of increased exophagy on EIR  
6 could be significant and of a magnitude comparable to, or exceeding, physiological resistance. There  
7 were also large differences in the predicted impact of resistance, particularly behavioural resistance,  
8 depending on the model assumptions regarding the structure of the mosquito population,  
9 specifically whether there is one homogenously mixed population or distinct populations of indoor  
10 and outdoor feeding mosquitoes. This preliminary modelling work highlights the importance of  
11 understanding mosquito behaviour.

### 13 **Is it all bad news?**

14 Causing vectors to feed more often outdoors may actually represent new opportunities for control.  
15 Blood-feeding vectors can be captured in odour-baited traps (Okumu et al. 2010), killed by  
16 insecticide-treated cattle (Rowland et al. 2001) or after feeding on attractive toxic sugar bait (Muller  
17 et al. 2010), whilst gravid females might be targeted if we can develop effective oviposition traps  
18 (Harris et al. 2011). It is essential that new tools continue to be developed targeting outdoor-feeding  
19 mosquitoes as their relative contribution to disease transmission will increase under successful LLINS  
20 and IRS campaigns. Behavioural changes favouring outdoor feeding and resting will also reduce  
21 vector exposure to insecticides inside the home, thereby reducing the selection pressure for  
22 physiological resistance.

24 The overall epidemiological effects of physiological insecticide resistance are not easy to estimate  
25 because the impact of an insecticide on individual mosquitoes is not only affected by genotype, but  
26 also their age and environment. Insecticide resistance is often strongest in young adults (Lines J.D.

and Nassor 1991, Rowland and Hemingway 1987). The use of LLINS and IRS results in few mosquitoes surviving to be old enough to transmit malaria parasites so any (resistance) gene that increases survival during the first one or two gonotrophic cycles will have a major positive selective advantage. If as mosquitoes age the survival benefits of the gene decrease, many resistant mosquitoes may die before reaching the minimum infectious age. Hence malaria is still controllable, albeit to a lesser extent than in a purely sensitive mosquito population.

A side effect of physiological resistance is often a reduction in the behavioural responsiveness to the insecticide (Hodjati and Curtis 1997, Rowland 1990). For example, in one study, pyrethroid resistant mosquitoes show reduced irritability when in contact with the insecticide causing them to rest on the surface for longer periods than susceptible mosquitoes, thus increasing the dose of insecticide received (Hodjati and Curtis 1997). In most cases the effect of physiological resistance is unquantified and dependent on the mechanism of resistance (Rivero et al. 2010). There has also been a recent suggestion that insecticides may select for vectors that invest in short-term reproduction rather than longer-term survival, resulting in a reduction in the number of older mosquitoes and a corresponding reduction in those able to transmit malaria parasites (Ferguson et al. 2012). For these reasons the overall consequences of accrued physiological and behavioural changes developed in response to the large-scale use of insecticides may not necessarily all be negative.

## **The way forward**

This review has highlighted a number of gaps in our knowledge of behavioural resistance in the vectors which transmit malaria; conclusive evidence for the evolution of behavioural resistance has often been confounded by methodological issues. However, our preliminary modelling study has demonstrated that behavioural resistance could have a significant impact on the effectiveness of malaria control. As a result, we propose seven recommendations to improve understanding of both physiological and behavioural resistance in malaria vectors.

- 1        1. Develop robust methodologies for detecting specific types of behavioural resistance in the
- 2        field.
- 3        2. Establish sentinel sites for long-term surveillance of physiological and behavioural resistance .
- 4        3. Improve understanding of the variability in behaviour of individuals within a larger
- 5        population of vectors (i.e. natural heterogeneity of population).
- 6        4. Report absolute mosquito abundance for each species in field studies, rather than reporting
- 7        only proportional changes.
- 8        5. Determine whether apparent cases of behavioural resistance are due to heritable traits, and
- 9        if so, develop diagnostic tests or identify a measured phenotype.
- 10       6. Better understand how physiological resistance may affect behaviour, and consequently
- 11       vectorial capacity.
- 12       7. Improve understanding of the behaviour of male mosquitoes relative to exposure to
- 13       insecticides via IRS and LLINS.

14

15    Determining the public health impact of both behavioural and physiological insecticide resistance is

16    an urgent priority if we are to maintain the significant gains that have been made in reducing malaria

17    morbidity and mortality over the past decade. While there is still much research needed to

18    understand better the spectrum of changes induced by intensive insecticide use, two points are

19    paramount for future policy discussions. First, it must be remembered that interventions such as

20    LLINs will provide some level of personal protection by presenting a physical barrier between

21    sleeping hosts and mosquitoes, irrespective of the level of resistance, provided they remain in good

22    condition. Therefore the development of insecticide resistance should never be a justification for

23    removing or reducing the distribution of LLINs; rather, additional or modified interventions should be

24    considered. Second, behavioural resistance cannot generally be addressed by simply changing

25    insecticides. Instead, novel interventions exploiting new behavioural patterns are required. It is not

26    unreasonable to recommend that interventions targeting outdoor feeding mosquitoes be the

- 1 mandatory second phase of all intervention programs given the probability that resistance will
- 2 eventually develop. At the moment this second phase is lacking from most intervention programs but
- 3 the time has come to correct this.
- 4

## **Authors' contributions**

All authors were involved in conception of this paper. MLG and SWL reviewed the literature and wrote the initial manuscript draft. All authors provided critical revisions.

## **Conflict of interest statements**

The authors have no competing interests.

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1    **Figure legends**

2    Figure 1. Distribution of biting times for *An. arabiensis* after 40 years of DDT IRS in northern Ethiopia  
3    (Yohannes et al. 2005) and after 8 years of DDT IRS in northern Sudan (Dukeen and Omer 1986).

4    Figure 2. Distribution of biting times for *An. farauti* before and after DDT spraying in the Solomon  
5    Islands (Taylor 1975).

6    Figure 3. Predicted average EIR for the first 10 years of vector control (LLINs and IRS) using two  
7    mathematical models (Imperial and OpenMalaria) of malaria transmission.

1    **Box 1** - Definitions

2    *Anthrophagy*: species that feed on humans mainly

3    *Contact irritant*: a chemical which stimulates mosquitoes to move away from the source after  
4        physical contact occurs

5    *Endophagy*: species that have a preference to feed indoors

6    *Endophily*: an inherent tendency to rest indoors after feeding (mosquitoes may feed indoors or  
7        outdoors)

8    *Exophagy*: species that have a preference to feed outdoors mainly

9    *Exophily*: species that have a preference to rest outdoors mainly

10   *Spatial repellent/deterrent*: a chemical which stimulates mosquitoes to move away from the source  
11        without the need for physical contact

12   *Toxic chemical action*: knockdown or death of mosquitoes after physical contact with the chemical

13   *Vectorial capacity*: the total number of infectious mosquito bites on humans that will arise from a  
14        single infected person on a single day

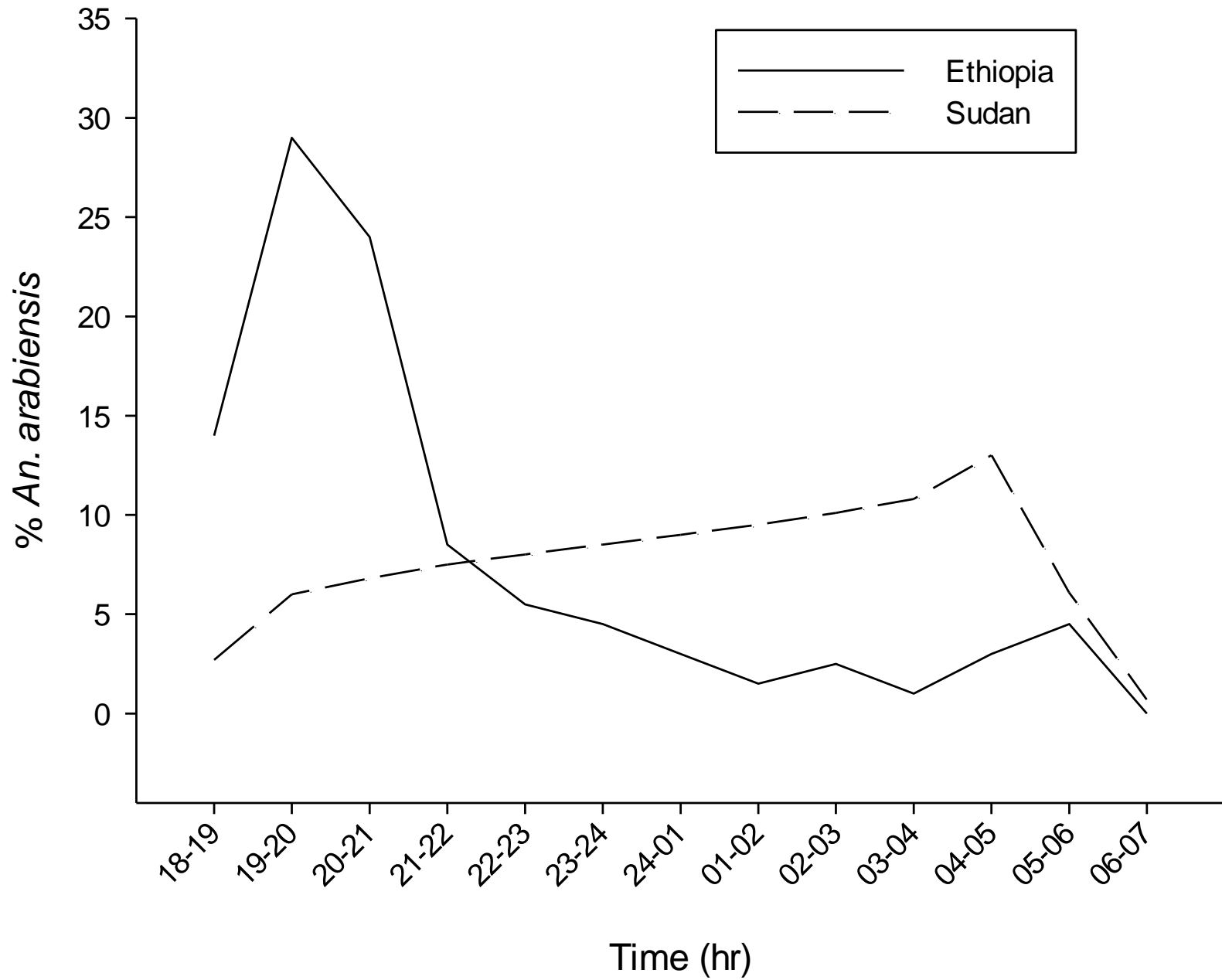
15   *Zoophagy*: species that feed on animals mainly

**Box 2 – Modelling insecticide resistance and its impact on a combined LLN - IRS intervention**

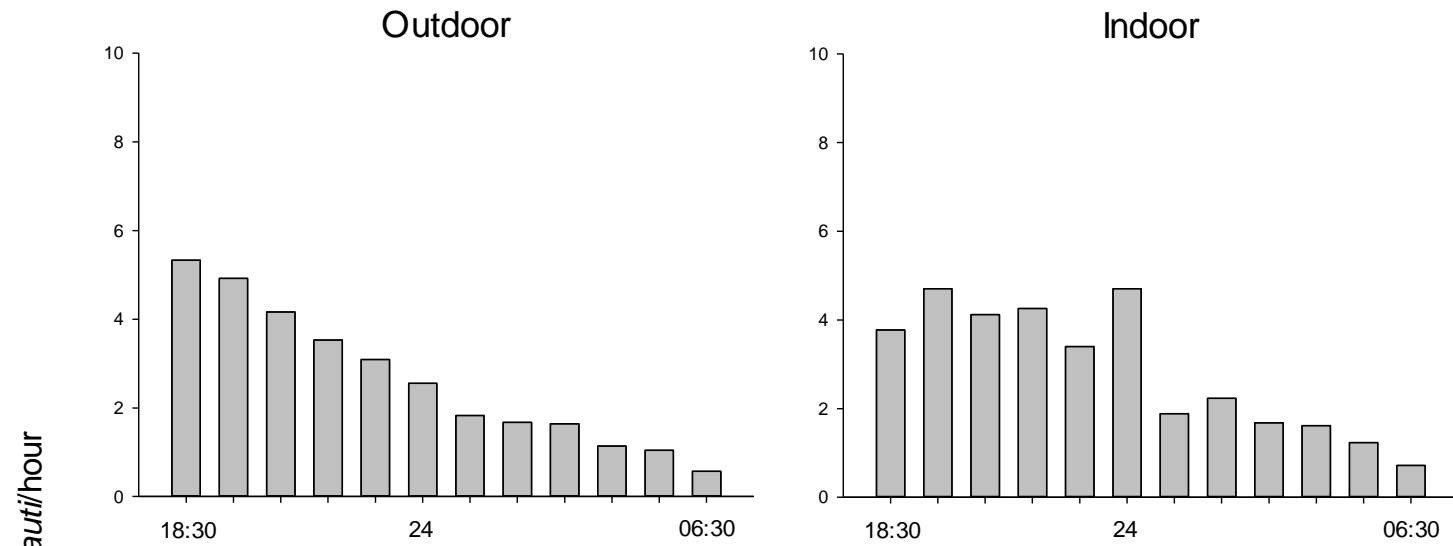
The potential impact of insecticide resistance, both behavioral and physiological, on malaria transmission was assessed using two independent mathematical models: Imperial (Griffin et al. 2010) and OpenMalaria (Chitnis et al. 2012, OpenMalaria 2012, Smith T. et al. 2008). Both models incorporated the full malaria transmission cycle by including the mosquito lifecycle as well as the human disease component. Three independent simulations were conducted using each model; 1) baseline simulation where 80% of the population used LLNs which were replaced every three years and pyrethroid IRS treatment was applied every year to 80% of houses (both distributed at random), 2) physiological resistance where the interventions were the same as the baseline simulation but the effects of the insecticide (both killing and repellency) were reduced by 70% (though the physical effects of the nets remained the same), and 3) behavioral resistance where the interventions were the same as the baseline simulation but exophagy of the vectors was increased so that 70% of bites take place outside, with all other parameters kept constant. The two models assumed extremes of mosquito biting behavior; the Imperial model assumed only one population of mosquitoes that sometimes bit outdoors and sometimes indoors, while the OpenMalaria model assumed two populations of mosquitoes that either always bit indoors or always outdoors (Molineaux et al. 1979). All simulations assumed a half-life of 3 months for pyrethroid effectiveness with an exponential decay. The models were parameterized for *An. gambiae* s.s. with no seasonality. All other baseline parameters were as previously reported for the individual models: Imperial (Griffin et al. 2010) and OpenMalaria (Briët et al. 2012).

All simulations were calibrated so that the average EIR was 100 infectious bites per person per year prior to the introduction of the LLNs and IRS interventions. To investigate the impact of insecticide resistance (rather than the spread), it was assumed that the physiological or behavioral resistance was present when the interventions were introduced.

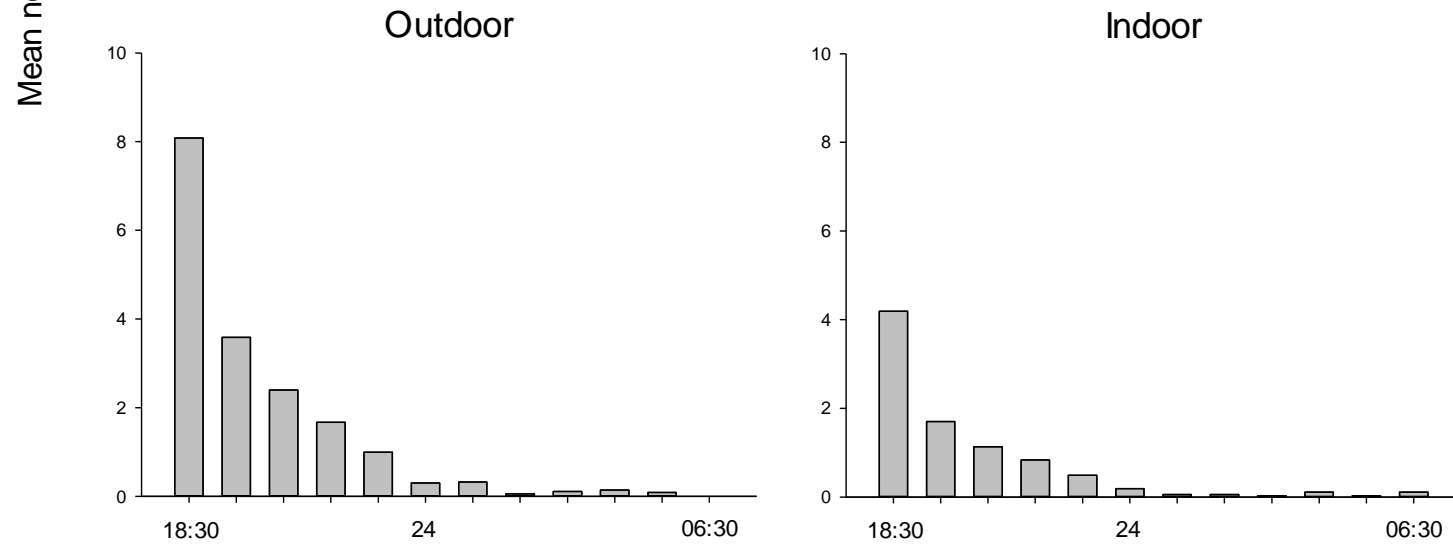
1 Ten years after the start of the interventions the average EIR in both models decreased by  
2 approximately 90% to 6.1 and 12.0 infectious bites per person per year using the Imperial model and  
3 the OpenMalaria model, respectively (Figure 3). As expected, the presence of physiological resistance  
4 reduced the impact of the interventions. Increased exophagy in the behavioral resistance simulations  
5 also decreased the effectiveness of the interventions; with the EIR predictions over 10 years after the  
6 start of the vector control being similar to, or higher than, those predicted for physiological  
7 resistance (Figure 3). This suggests that the impacts of behavioral resistance could potentially be as  
8 severe, or even worse than, those of physiological resistance.



# BEFORE SPRAY



# AFTER SPRAY



Time



